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(54) Title: METHODS FOR USING RESONANCE ENERGY TRANSFER-BASED ASSAY OF HIV-1 ENVELOPE CLYCOPROTEIN-MEDIATED MEMBRANE FUSION, AND KITS FOR PRACTICING SAME

(57) Abstract

The subject invention provides methods for determining whether an agent is capable of either inhibiting or specifically inhibiting the fusion of a CD4+ cell with an HIV-1 envelope glycoprotein+cell. The subject invention also provides a method for determining whether an agent is capable of specifically inhibiting the infection of a CD4+cell with HIV-1. This invention also provides methods for quantitatively determining the ability of an antibody-containing sample to either inhibit or specifically inhibit the fusion of CD4+cell with an HIV-1 envelope glycoprotein+cell.

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METHODS FOR USING RESONANCE ENERGY TRANSFER-BASED ASSAY OF HIV-1 ENVELOPE GLYCOPROTEIN-MEDIATED MEMBRANE FUSION, AND KITS FOR PRACTICING SAME

Background of the Invention

Throughout this application, various publications are referenced. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the art to which this invention pertains.

HIV infects primarily helper T lymphocytes and monocytes/macrophages--cells that express surface CD4--leading to a gradual loss of immune function which results in the development of the human acquired immune deficiency syndrome (AIDS). The initial phase of the HIV replicative cycle involves the high affinity interaction between the HIV exterior envelope glycoprotein gp120 and the cellular receptor CD4 (Klatzmann, D.R., et al., Immunodef. Rev. 2, 43-66 (1990)). Following the attachment of HIV to the cell surface, viral and target cell membranes fuse, resulting in the introduction of the viral genome into the cytoplasm. Several lines of evidence demonstrate the requirement of this interaction for viral infectivity. In vitro, the introduction of a functional cDNA encoding CD4 into human cells which do not normally express CD4 is sufficient to render these otherwise resistant cells susceptible to HIV infection (Maddon, P.J., et al., Cell 47, 333-348 (1986)).

Characterization of the interaction between HIV gp120 and CD4 has been facilitated by the isolation of cDNA clones encoding both molecules (Maddon, P.J., et al., Cell 42, 93-104 (1985), Wain-Hobson, S., et al., Cell 40, 9-17

(1985)). CD4 is a nonpolymorphic, lineage-restricted cell surface glycoprotein that is a member of the immunoglobulin gene superfamily. High-level expression of both full-length and truncated, soluble versions of CD4 (sCD4) have been described in stable expression systems. The availability of large quantities of purified sCD4 has permitted a detailed understanding of the structure of this complex glycoprotein. Mature CD4 has a relative molecular weight of 55,000 and consists of an amino-terminal 372 amino acid extracellular domain containing four tandem immunoglobulin-like regions denoted V1-V4, followed by a 23 amino acid transmembrane domain and a 38 amino acid cytoplasmic segment. Experiments using truncated sCD4 proteins demonstrate that the determinants of high-affinity binding to HIV gp120 lie within the amino-terminal immunoglobulin-like domain V1 (Arthos, J., et al., Cell 57, 469-481 (1989)). Mutational analysis of V1 has defined a discrete gp120-binding site (residues 38-52 of the mature CD4 protein) that comprises a region structurally homologous to the second complementarity-determining region (CDR2) of immunoglobulins (Arthos, J., et al., Cell 57, 469-481 (1989)).

The HIV-1 envelope gene env encodes an envelope glycoprotein precursor, gp160, which is cleaved by cellular proteases before transport to the plasma membrane to yield gp120 and gp41. The membrane-spanning glycoprotein, gp41, is non-covalently associated with gp120, a purely extracellular glycoprotein. The mature gp120 molecule is heavily glycosylated (approximately 24 N-linked oligosaccharides), contains approximately 480 amino acid residues with 9 intra-chain disulfide bonds (Leonard, C.K., et. al., J. Biol. Chem. 265, 10373-10382 (1990)), and projects from the viral membrane as a

dimeric or multimeric molecule (Earl, P.L., et. al. Proc. Natl. Acad. Sci. U.S.A. 87, 648-652 (1990)).

Mutational studies of HIV-1 gp120 have delineated important functional regions of the molecule. The regions of gp120 that interact with gp41 map primarily to the N- and C- termini (Helseth, E., et. al., J. Virol. 65, 2119-2123 (1991)). The predominant strain-specific neutralizing epitope on gp120 is located in the 32-34 amino acid residue third variable loop, herein referred to as the V3 loop, which resides near the center of the gp120 sequence (Bolognesi, D.P. TIBTech 8, 40-45 (1990)). The CD4-binding site maps to discontinuous regions of gp120 that include highly conserved or invariant amino acid residues in the second, third, and fourth conserved domains (the C2, C3 and C4 domains) of gp120 (Olshevsky, U., et al. J. Virol. 64, 5701-5707 (1990)). It has been postulated that a small pocket formed by these conserved residues within gp120 could accommodate the CDR2 loop of CD4, a region defined by mutational analyses as important in interacting with gp120 (Arthos, J., et al., Cell 57, 469-481 (1989)).

Following the binding of HIV-1 gp120 to cell surface CD4, viral and target cell membranes fuse, resulting in the introduction of the viral capsid into the target cell cytoplasm (Maddon, P.J. et al., Cell 54:865 (1988)). Most evidence to date indicates that HIV-1 fusion is pH-independent and occurs at the cell surface. The HIV-1 fusion protein is gp41, the transmembrane component of the envelope glycoprotein. This protein has a hydrophobic fusion peptide at the amino-terminus and mutations in this peptide inhibit fusion (Kowalski, M. et al., Science 237:1351 (1987)). In addition to gp41, recent observations suggest that gp120 plays a role in

membrane fusion distinct from its function in attachment. For example, antibodies to the principle neutralizing epitope on gp120, the V3 loop, can block infection without inhibiting attachment (Skinner, M.A. et al., J. Virol. 62:4195 (1988)). in addition, mutations in the tip of this loop reduce or prevent syncytia formation in HeLa-CD4 cells expressing the mutated gp120/gp41 molecules (Freed, E.O. et al., J. Virol. 65:190 (1991)).

Several lines of evidence have implicated molecules in addition to CD4 and gp120/gp41 in HIV-1 induced membrane fusion. For example, recent studies have indicated that human cells may contain an accessory molecule, not present in non-primate cells, which is required for HIV-1 fusion (Dragic, T. et al., J. Virol. 66:4794 (1992)). The nature of this accessory molecule or molecules is unknown. While some studies have postulated it might be a cell surface protease (Hattori, T. et al., Febs. Lett. 248:48 (1989)), this has yet to be confirmed.

Fusion of the HIV-1 virion with the host cell plasma membrane is mimicked in many ways by the fusion of HIV-1 infected cells expressing gp120/gp41 with uninfected cells expressing CD4. Such cell-to-cell fusion results in the formation of multinucleated giant cells or syncytia, a phenomenon observed with many viruses which fuse at the cell surface. Much of our current understanding of HIV-1-induced membrane fusion is derived from studies of syncytium formation. For example, this approach was used to demonstrate that expression of HIV-1 gp120/gp41 in a membrane, in the absence of any other viral protein, is necessary and sufficient to induce fusion with a $CD4^{+}$ membrane (Lifson, J.D. et al., Nature 323:725 (1986)).

Compared with virion fusion to cells, syncytium formation induced by HIV-1 appears to involve an additional step. First, the gp120/gp41-bearing membrane fuses with the CD4- bearing membrane. This is a rapid and reversible process which connects the membranes at localized sites and allows membrane-bound dyes to flow from one cell to the other (Dimitrov, D. et al., AIDS Res. Human Retroviruses 7:799 (1991)). This step presumably parallels the attachment of a virion to a $CD4^{+}$ cell and the fusion therebetween. The second stage in cells fusion is the irreversible fusion of cells to form syncytia. The efficiency of this process is increased by the interaction of cellular adhesion molecules such as ICAM-1 and LFA-1, although these molecules are not absolutely required for syncytium formation to proceed (Golding, H. et al., AIDS Res. Human Retroviruses 8:1593 (1992)).

Most of the studies of HIV-1 fusion, including those discussed above, have been performed with strains of HIV-1 which have been extensively propagated in transformed human T cell lines. These strains, known as laboratory-adapted strains, differ in several important characteristics from primary or clinical isolates of the virus obtained from HIV-1 infected individuals (O' Brien, W.A. et al., Nature 348:69 (1990)). Some examples of these differences are listed in the table below.

Laboratory adapted Strains	Primary Isolates
tropic for transformed T cell lines, do not infect primary monocytes	many are tropic for primary monocytes and do not infect transformed T cell lines
very sensitive to neutralization by soluble CD4	relatively insensitive to neutralization by sCD4
gp120 spontaneously dissociates from gp41, and this stripping is increased by sCD4	little spontaneous stripping and sCD4 only causes stripping at 4°C, not at 37°C

These differences are mirrored by differences in the primary sequence of the viral proteins, and in particular of the envelope glycoproteins. In some cases, the different tropisms of primary isolates and laboratory-adapted strains of HIV-1 have been mapped to regions on gp120 such as the V3 loop (O' Brien, W.A. et al., Nature 348:69 (1990)). It is possible that different V3 loops interact with different accessory molecules on T cell lines or monocytes, thereby mediating tropism.

HIV-1 envelope-mediated cell fusion is a model for the early stages of HIV-1 infection and can be used as an assay for anti-viral molecules which block HIV-1 attachment and fusion (Sodroski, J. et al., Nature 322-470 (1986), Lifson, J.D. et al., Nature 323:725 (1986)). Moreover, HIV-1 induced cell fusion is important in its own right as a potential mechanism for the pathogenesis of HIV-1 infections. It is a mode of transmission of HIV-1 from infected to uninfected cells (Gupta, P. et al., J. Virol. 63:2361 (1989), Sato, H. et al., Virology 186:712 (1992)) and by this mechanism, it could contribute to the spread of HIV-1 throughout the body of

the infected individual. Cell fusion is also a direct mechanism of HIV-1-induced cell death (Sodroski, J. et al., Nature 322:470 (1986), Lifson, J.D. et al., Nature 323:725 (1986)). Syncytia are seen in vivo, notably in the brains of AIDS patients suffering from neurological complications such as AIDS dementia complex (Pumarola-Sune, T. et al., Ann. Neurol. 21:490 (1987)). In addition, syncytia have been observed in the spleens of HIV-1-infected individuals (Byrnes, R.K. et al., JAMA 250:1313 (1983)). It is possible that cell fusion may play a role in the depletion of $CD4^+$ T lymphocytes that is characteristic of the pathogenic process leading to AIDS (Haseltine, W.A. in AIDS and the new viruses, Dalgleish, A.G. and Weiss, R.A. eds. (1990)).

In this context, it may be significant that HIV-1 isolates from asymptomatic HIV-1-infected individuals often infect cells in vitro without inducing syncytia. In contrast, clinical isolates from patients with ARC and AIDS are commonly highly virulent, syncytia-inducing strains (Tersmette, M. et al., J. Virol. 62:2026 (1988)). In addition, there is often a switch from non-syncytium inducing (NSI) to syncytium-inducing (SI) isolates within patients as the disease progresses and symptoms appear (Tersmette, M. et al., J. Virol. 63:2118 (1989), Cheng-Mayer, C. et al., science 240:80 (1988)). It is not clear why some HIV-1 strains do not induce syncytia, although it is possible that cells infected with these strains do not express sufficient levels of gp120/gp41 for cell fusion to occur, by analogy with some other fusogenic viruses. However, it is believed that this switch from NSI to SI HIV-1 strains influences the clinical course of HIV-1 infection. The presence of naturally occurring anti-syncytia antibodies in some subjects may delay the development of HIV-1 related

diseases in these subjects (Brenner, T.J. et al., Lancet 337:1001 (1991)).

The development of methods for measuring HIV-1 envelope glycoprotein-mediated membrane fusion serves a useful role in further elucidating the mechanism of HIV-1 infection, and enabling the identification of agents which alter HIV-1 envelope glycoprotein-mediated cell fusion. At present there exist several potential methods for measuring such fusion.

The first is an assay of HIV-1 envelope glycoprotein-mediated cell fusion in which fusion is measured microscopically by measuring the transfer of fluorescent dyes between cells (Dimitrov, D.S., et al., AIDS Res. Human Retroviruses 7: 799-805 (1991)). This technique measures dye distribution rather than fluorescence intensity and as such cannot be performed using fluorometer. The assay would not be easily automated and has not been performed with cells which stably express the HIV-1 envelope glycoprotein.

The second is an assay for HIV-1 envelope-mediated cell fusion measured between (a) cells which stably express the HIV-1 tat gene product in addition to gp120/gp41, and (b) $CD4^+$ cells which contain a construct consisting of the β-galactosidase gene under the control of the HIV-1 LTR promotor. When these cells fuse, β-galactosidase is expressed and can be measured using an appropriate soluble or insoluble chromogenic substrate (Dragic, T., et al., Journal of Virology 66:4794 (1992)). This assay takes at least 1 day to perform and cannot easily be adapted to new target cells such as primary macrophage cells. This assay also does not measure cell fusion in real time and is thus not amenable to use in analyzing

fusion kinetics.

Finally, the third is a fluorescence dequenching assay for the fusion of HIV-1 virions to cells (Sinangil, F., et al., FEBS Letters 239:88-92 (1988)). This assay requires the use of purified HIV-1 virions, and both the purification of HIV-1 virions and the assay must be performed in a containment facility. It would be difficult to readily isolate sufficient quantities of clinical virus isolates to perform the assay. Furthermore, this assay is more complicated and less reproducible than a RET assay using cells which stably express HIV-1 envelope glycoproteins and CD4.

The methods of the subject invention employ a resonance energy transfer (RET) based assay which overcomes the problems inherent in the above-identified methods for measuring HIV-1 envelope glycoprotein-mediated membrane fusion. Specifically, the methods of the subject invention employ a RET assay which is rapid, reproducible, quantitative, adaptable to various cell types, and relatively safe, and can be automated.

Summary of the Invention

The subject invention provides a method for determining whether an agent is capable of specifically inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises: (a) contacting a sample containing a suitable amount of the agent with a suitable amount of the $CD4^+$ cell and a suitable amount of the HIV-1 envelope glycoprotein$^+$ cell under conditions which would permit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell in the absence of the agent, the cell membranes of the $CD4^+$ cell and the HIV-1 envelope glycoprotein$^+$ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to determine whether the agent is capable of inhibiting fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell; and (d) determining whether the agent inhibits the fusion of a first control cell with a second control cell under conditions which would permit non-HIV-1 envelope glycoprotein-mediated fusion of the first and second control cells in the absence of the agent, so as to determine whether the agent is capable of specifically inhibiting the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell.

The subject invention also provides a method for determining whether an agent is capable of specifically inhibiting the infection of a $CD4^+$ cell with HIV-1

which comprises determining whether the agent is capable of specifically inhibiting the fusion of a CD4^{+} cell with an HIV-1 envelope glycoprotein^{+} cell by the method of the subject invention, so as to thereby determine whether the agent is capable of specifically inhibiting the infection of a CD4^{+} cell with HIV-1.

The subject invention further provides a method for determining whether an agent is capable of inhibiting the fusion of a CD4^{+} cell with an HIV-1 envelope glycoprotein^{+} cell which comprises: (a) contacting a sample containing a suitable amount of the agent with a suitable amount of the CD4^{+} cell and a suitable amount of the HIV-1 envelope glycoprotein^{+} cell under conditions which would permit the fusion of the CD4^{+} cell with the HIV-1 envelope glycoprotein^{+} cell in the absence of the agent, the cell membranes of the CD4^{+} cell and the HIV-1 envelope glycoprotein^{+} cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to determine whether the agent is capable of inhibiting fusion of the CD4^{+} cell with the HIV-1 envelope glycoprotein^{+} cell.

This invention also provides an agent determined by the above-described method.

The subject invention further provides a method for quantitatively determining the ability of an antibody-containing sample to specifically inhibit the fusion of a CD4^{+} cell with an HIV-1 envelope glycoprotein^{+} cell

which comprises: (a) contacting a predetermined amount of the antibody-containing sample with a suitable amount of the CD4⁺ cell and a suitable amount of the HIV-1 envelope glycoprotein⁺ cell under conditions which would permit the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell in the absence of the antibody-containing sample, the cell membranes of the CD4⁺ cell and the HIV-1 envelope glycoprotein⁺ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to quantitatively determine the ability of the antibody-containing sample to inhibit the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell; and (d) determining whether the antibody-containing sample inhibits the fusion of a first control cell with a second control cell under conditions which would permit non-HIV-1 envelope glycoprotein-mediated fusion of the first and second control cells in the absence of the agent, so as to quantitatively determine the ability of the antibody-containing sample to specifically inhibit the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell.

The subject invention further provides a method for quantitatively determining the ability of an antibody-containing sample to inhibit the fusion of a CD4⁺ cell with an HIV-1 envelope glycoprotein⁺ cell which comprises: (a) contacting a predetermined amount of the antibody-containing sample with a suitable amount of the CD4⁺ cell and a suitable amount of the HIV-1 envelope glycoprotein⁺

cell under conditions which would permit the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell in the absence of the antibody-containing sample, the cell membranes of the CD4⁺ cell and the HIV-1 envelope glycoprotein⁺ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to quantitatively determine the ability of the antibody-containing sample to inhibit the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell.

The subject invention further provides a method for determining the stage or clinical prognosis of an HIV-1 infection in an HIV-1-infected subject which comprises: (a) obtaining an antibody-containing sample from the HIV-1-infected subject; (b) quantitatively determining the ability of the antibody-containing sample so obtained to inhibit the fusion of a CD4⁺ cell with an HIV-1 envelope glycoprotein⁺ cell by the method of the subject invention; and (c) comparing the ability of the antibody-containing sample to inhibit the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell so determined with that of an antibody-containing sample obtained from an HIV-1-infected subject having an HIV-1 infection at a known stage or having a known clinical prognosis, so as to determine the stage or clinical prognosis of the HIV-1 infection in the HIV-1-infected subject.

The subject invention further provides a method for determining the efficacy of an anti-HIV-1 vaccination in

a vaccinated, non-HIV-1-infected subject which comprises: (a) obtaining an antibody-containing sample from the vaccinated, non-HIV-1-infected subject; (b) quantitatively determining the ability of the antibody-containing sample so obtained to inhibit the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell by the method of the subject invention; and (c) comparing the ability of the antibody-containing sample to inhibit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell so determined with that of an antibody-containing sample obtained from a vaccinated, non-HIV-1-infected subject for whom the anti-HIV-1 vaccination has a known efficacy, so as to determine the efficacy of the anti-HIV-1 vaccination in the vaccinated, non-HIV-1-infected subject.

The subject invention further provides a kit for determining whether an agent is capable of specifically inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises, in separate compartments: (a) a suitable amount of a $CD4^+$ cell whose cell membrane is labeled with a first dye; (b) a suitable amount of an HIV-1 envelope glycoprotein$^+$ cell whose cell membrane is labeled with a second dye, the HIV-1 envelope glycoprotein$^+$ cell being capable of fusing with the $CD4^+$ cell of (a) under suitable conditions in the absence of the agent, and the first and second dyes permitting resonance energy transfer therebetween only when juxtaposed within the same membrane; (c) a suitable amount of a first control cell whose cell membrane is labeled with the first dye; and (d) a suitable amount of a second control cell whose cell membrane is labeled with the second dye, the second control cell being capable of non-HIV-1 envelope glycoprotein-mediated fusion with the first control cell of (c) under suitable conditions in

the absence of the agent.

The subject invention further provides a kit for determining whether an agent is capable of inhibiting the fusion of a CD4⁺ cell with an HIV-1 envelope glycoprotein⁺ cell which comprises, in separate compartments: (a) a suitable amount of a CD4⁺ cell whose cell membrane is labeled with a first dye; and (b) a suitable amount of an HIV-1 envelope glycoprotein⁺ cell whose cell membrane is labeled with a second dye, the HIV-1 envelope glycoprotein⁺ cell being capable of fusing with the CD4⁺ cell of (a) under suitable conditions in the absence of the agent, and the first and second dyes permitting resonance energy transfer therebetween only when juxtaposed within the same membrane.

The subject invention further provides a method for determining whether an HIV-1 isolate is syncytium-inducing which comprises: (a) obtaining a sample of an HIV-1 isolate envelope glycoprotein⁺ cell whose cell membrane is labeled with a first dye; (b) contacting a suitable amount of the sample with a suitable amount of a CD4⁺ cell under conditions which would permit the fusion of the CD4⁺ cell with a syncytium-inducing HIV-1 strain envelope glycoprotein⁺ cell, the cell membrane of the CD4⁺ cell being labeled with a second dye which permits resonance energy transfer between the first dye only when the first and second dyes are juxtaposed within the same membrane; (c) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and (d) comparing the percent resonance energy transfer value so determined with a known standard, so as to determine whether the HIV-1 isolate is syncytium-inducing.

Finally, the subject invention provides a method for determining the stage of an HIV-1 infection in an HIV-1-infected subject which comprises determining by the method of the subject invention whether the HIV-1 isolate with which the HIV-1 infected subject is infected is syncytium inducing, so as to thereby determine the stage of the HIV-1 infection in the HIV-1-infected subject.

Brief Description of the Figures

Figure 1

Time course of fusion between HeLa-env^{+} cells and HeLa-CD4^{+} cells measured by the RET assay.

Figure 2

Blocking of fusion between HeLa-env^{+} cells and HeLa-CD4^{+} cells by OKT4a, measured using RET.

Figure 3

Blocking of fusion between 160G7 cells and C8166 cells by sCD4, measured using RET.

Figure 4

A comparative analysis of results of blocking experiments by two methods using OKT4a to inhibit the fusion of HeLa-env^{+} and HeLa-CD4^{+} cells.

Detailed Description of the Invention

The plasmid designated pMA243 was deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 under ATCC Accession No. 75626. The plasmid pMA243 was deposited with the ATCC on December 16, 1993.

The subject invention provides a method for determining whether an agent is capable of specifically inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises: (a) contacting a sample containing a suitable amount of the agent with a suitable amount of the $CD4^+$ cell and a suitable amount of the HIV-1 envelope glycoprotein$^+$ cell under conditions which would permit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell in the absence of the agent, the cell membranes of the $CD4^+$ cell and the HIV-1 envelope glycoprotein$^+$ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to determine whether the agent is capable of inhibiting fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell; and (d) determining whether the agent inhibits the fusion of a first control cell with a second control cell under conditions which would permit non-HIV-1 envelope glycoprotein-mediated fusion of the first and second

control cells in the absence of the agent, so as to determine whether the agent is capable of specifically inhibiting the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell.

This invention provides an agent determined to be capable of specifically inhibiting the fusion of a CD4⁺ cell with an HIV-1 envelope glycoprotein⁺ cell using the above-described method.

As used herein, the term "agent" includes both protein and non-protein moieties. In one embodiment, the agent is a small molecule. In another embodiment, the agent is a protein. The protein may be, by way of example, an antibody directed against a portion of an HIV-1 envelope glycoprotein, e.g., gp120. The agent may be derived from a library of low molecular weight compounds or a library of extracts from plants or other organisms.

As used herein, "capable of specifically inhibiting the fusion of a CD4⁺ cell with an HIV-1 envelope glycoprotein⁺ cell" means (a) capable of reducing the rate of fusion of CD4⁺ cell membrane with HIV-1 envelope glycoprotein⁺ cell membrane by at least 5%, but not capable of reducing the rate of non-CD4/HIV-1 envelope glycoprotein-mediated cell membrane fusion, or (b) capable of reducing by at least 5% the total amount of fusion of CD4⁺ cell membrane with HIV-1 envelope glycoprotein⁺ cell membrane occurring by the endpoint of fusion, but not capable of reducing the total amount of non-CD4/HIV-1 envelope glycoprotein-mediated cell membrane fusion occurring by the endpoint of fusion. As used herein, the rate of cell membrane fusion means the total quantity of cell membrane fused per unit of time. As used herein, the "endpoint of fusion" means the point in time at which all fusion of

$CD4^+$ cell membrane with HIV-1 envelope glycoprotein$^+$ cell membrane capable of occurring has occurred.

An example of the method of the subject invention is provided infra. A known amount of $CD4^+$ cell is contacted with a known amount of HIV-1 envelope glycoprotein$^+$ cell together with an agent under conditions which would permit the fusion of Y amount of cell membrane per unit of time in the absence of the agent, wherein Y is equal to the sum of the amounts of $CD4^+$ cell membrane and HIV-1 envelope glycoprotein$^+$ cell membrane, e.g., 0.5 x Y $CD4^+$ cell membrane + 0.5 x Y HIV-1 envelope glycoprotein$^+$ cell membrane. In the presence of the agent, 0.2 x Y amount of cell membrane fuses per unit of time. The agent is shown not to reduce the rate of non-CD4/HIV-1 envelope glycoprotein-mediated cell membrane fusion. Accordingly, the agent specifically inhibits the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell.

As used herein, the fusion of $CD4^+$ cell membrane with HIV-1 envelope glycoprotein$^+$ cell membrane means the hydrophobic joining and integration of $CD4^+$ cell membrane with HIV-1 envelope glycoprotein$^+$ cell membrane to form a hybrid membrane comprising components of both cell membranes, and does not mean the CD4/HIV-1 envelope glycoprotein-mediated adhesion therebetween, which adhesion is a prerequisite for the fusion.

As used herein, the term "CD4" includes (a) native CD4 protein and (b) a membrane-bound CD4-based protein. As used herein, a membrane-bound CD4-based protein is any membrane-bound protein, other than native CD4, which comprises at least that portion of native CD4 which is required for native CD4 to form a complex with the HIV-1 gp120 envelope glycoprotein. In one embodiment, the CD4-

based protein comprises a portion of a non-CD4 protein. If the CD4-based protein comprises a portion of a non-CD4 protein, then the portion of native CD4 which is required for native CD4 to form a complex with the HIV-1 gp120 envelope glycoprotein is the portion of native CD4 having the amino acid sequence from +1 to about +179.

As used herein, the word "cell" includes a biological cell, e.g., a HeLa cell, and a non-biological cell, e.g., a lipid vesicle (e.g., a phospholipid vesicle) or virion.

As used herein, a CD4$^+$ cell is a cell having CD4 affixed to the surface of its cell membrane, wherein the CD4$^+$ cell is capable of specifically binding to and fusing with an HIV-1 envelope glycoprotein$^+$ cell exposed thereto. In the preferred embodiment, the suitable CD4$^+$ cell is a CD4$^+$ HeLa cell.

As used herein, an HIV-1 envelope glycoprotein$^+$ cell is a cell having HIV-1 envelope glycoprotein affixed to the surface of its cell membrane so as to permit the HIV-1 envelope glycoprotein$^+$ cell to specifically bind to and fuse with a CD4$^+$ cell exposed thereto. In one embodiment, the HIV-1 envelope glycoprotein$^+$ cell is an HIV-1 envelope glycoprotein$^+$ HeLa cell. In another embodiment, the HIV-1 envelope glycoprotein$^+$ cell is HIV-1.

Each HIV-1 isolate is tropic for a limited number of CD4$^+$ cell types. Accordingly, in the subject invention, the fusion of a CD4$^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell means the fusion of a CD4$^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell, which HIV-1 envelope glycoprotein corresponds to an envelope glycoprotein from an HIV-1 isolate tropic for the CD4$^+$ cell. For example, the HIV-1 isolates JR-FL, JR-CSF and BaL are tropic for

$CD4^+$ primary human macrophages, the HIV-1 isolates LAI and IIIB are tropic for human $CD4^+$ T lymphocyte cell lines and HeLa-CD4 cells, and the HIV-1 isolates MN and SF-2 are tropic for human $CD4^+$ T lymphocyte cell lines. The HIV-1 isolates JR-FL, JR-CSF, BaL, LAI, IIIB, MN and SF-2 may also be tropic for $CD4^+$ cell types other than those enumerated supra.

The suitable amounts of agent, $CD4^+$ cell and HIV-1 envelope glycoprotein$^+$ cell may be determined according to methods well known to those skilled in the art.

Conditions which would permit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell in the absence of the agent are well known to those skilled in the art.

As used herein, a cell "labeled" with a dye means a cell having a dye integrated into its cell membrane, i.e., a cell having dye molecules commingled with the lipid molecules of its cell membrane.

Resonance energy transfer is defined as follows: For juxtaposed dyes D1, having excitation and emission spectra Ex1 and Em1, respectively, and D2, having excitation and emission spectra Ex2 and Em2, respectively, wherein (a) Em1 has a higher average frequency than that of Em2 and (b) Em1 and Ex2 overlap, resonance energy transfer is the transfer of electromagnetic energy from D1 to D2 at a frequency within the Em1 and Ex2 overlap, which resonance energy transfer (a) results from the electromagnetic excitation of D1 at a frequency within the Ex1 spectrum and (b) causes the subsequent emission of electromagnetic energy from D2 at a frequency within the Em2 spectrum. Accordingly, resonance energy transfer between D1 and D2

can be detected by exciting D1 with electromagnetic energy at a frequency within Ex1 and measuring the subsequently emitted electromagnetic energy at a frequency within Em2, the emission of electromagnetic energy at a frequency within Em2 indicating the occurrence of resonance energy transfer between D1 and D2.

The first and second dyes are "juxtaposed within the same membrane" if they are present within the same lipid membrane at a suitably short distance from each other, which suitably short distance may be readily determined by one skilled in the art.

In the subject invention, determining the percent resonance energy transfer value may be performed according to methods well known to those skilled in the art. In one embodiment, the percent resonance energy transfer value is determined by: (1) determining the resonance energy transfer value (RET) by subtracting from the total emission from D1 and D2 at a frequency within Em2 the electromagnetic energy emission due to direct D1 and D2 emission following excitation at a frequency within Ex1 and emission at the frequency within Em2, which D1 and D2 emissions are measured by separately measuring the electromagnetic energy emission due to cells labeled with each dye; and (2) determining the percent resonance energy transfer value (% RET value) by dividing the resonance energy transfer value obtained in step (1) by the total D2 emission at the frequency within Em2.

The suitable period of time after which the percent resonance energy transfer value of the resulting sample is determined may be determined according to methods well

known to those skilled in the art.

The known standard is a percent resonance energy transfer value obtained using the $CD4^+$ cell, the HIV-1 envelcpe glycoprotein$^+$ cell, and an agent having a known ability to inhibit the fusion thereof.

In the subject invention, the first control cell and second control cell are capable of fusing with each other via non-HIV-1 envelope glycoprotein-mediated fusion both in the presence and absence of an agent capable of inhibiting HIV-1 envelope glycoprotein-mediated fusion, and are not capable of fusing via HIV-1 envelope glycoprotein-mediated fusion. Such cells are will known to those skilled in the art, and include, by way of example, HeLa cells which can be induced to fuse with each other by incubation at 37°C with polyethylene glycol 1000 or with Sendai virus. These methods of inducing fusion of HeLa cells are well known to those skilled in the art.

In one embodiment, the agent is an antibody. As used in the subject invention, the term "antibody" includes, but is not limited to, both naturally occurring and non-naturally occurring antibodies. Specifically, the term "antibody" includes polyclonal and monoclonal antibodies, and antigen-binding fragments thereof. Furthermore, the term "antibody" includes chimeric antibodies, wholly synthetic antibodies, and antigen-binding fragments thereof.

In one embodiment, the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule. Rhodamine moiety-containing molecules and fluorescein moiety-containing molecules are

well known to those skilled in the art.

In the preferred embodiment, the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

In another embodiment, the first dye is a fluorescein moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

In one embodiment, the $CD4^+$ cell is a $CD4^+$ HeLa cell. In another embodiment, the HIV-1 envelope glycoprotein$^+$ cell is an HIV-1_{LAI} gp120/gp41$^+$ HeLa cell. HIV-1_{LAI} is a laboratory-adapted strain that is tropic for phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes (PBLs) and immortalized human T-cell lines.

The subject invention also provides a method for determining whether an agent is capable of specifically inhibiting the infection of a $CD4^+$ cell with HIV-1 which comprises determining whether the agent is capable of specifically inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell by the method of the subject invention, so as to thereby determine whether the agent is capable of specifically inhibiting the infection of a $CD4^+$ cell with HIV-1.

The subject invention further provides a method for determining whether an agent is capable of inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises: (a) contacting a sample containing a suitable amount of the agent with a suitable amount of the $CD4^+$ cell and a suitable amount of the HIV-1 envelope glycoprotein$^+$ cell under conditions which would permit the

fusion of the $CD4^{+}$ cell with the HIV-1 envelope glycoprotein^{+} cell in the absence of the agent, the cell membranes of the $CD4^{+}$ cell and the HIV-1 envelope glycoprotein^{+} cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to determine whether the agent is capable of inhibiting fusion of the $CD4^{+}$ cell with the HIV-1 envelope glycoprotein^{+} cell.

As used herein, "capable of inhibiting the fusion of a $CD4^{+}$ cell with an HIV-1 envelope glycoprotein^{+} cell" means capable of (a) reducing the rate of fusion of $CD4^{+}$ cell membrane with HIV-1 envelope glycoprotein^{+} cell membrane by at least 5%, or (b) reducing by at least 5% the total amount of fusion of $CD4^{+}$ cell membrane with HIV-1 envelope glycoprotein^{+} cell membrane occurring by the endpoint of fusion. An agent capable of inhibiting the fusion of a $CD4^{+}$ cell with an HIV-1 envelope glycoprotein^{+} cell may also be capable of reducing the rate to non-CD4/HIV-1 envelope glycoprotein-mediated cell membrane fusion.

This invention provides an agent determined to be capable of inhibiting the fusion of a $CD4^{+}$ cell with an HIV-1 envelope glycoprotein^{+} cell using the above-described method.

In one embodiment, the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule.

In the preferred embodiment, the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

In another embodiment, the first dye is a fluorescein moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

In one embodiment, the $CD4^+$ cell is a $CD4^+$ HeLa cell. In another embodiment of the subject invention, the HIV-1 envelope glycoprotein$^+$ cell is an HIV-1_{LAI} gp120/gp41$^+$ HeLa cell.

The subject invention further provides a method for quantitatively determining the ability of an antibody-containing sample to specifically inhibit the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises: (a) contacting a predetermined amount of the antibody-containing sample with a suitable amount of the $CD4^+$ cell and a suitable amount of the HIV-1 envelope glycoprotein$^+$ cell under conditions which would permit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell in the absence of the antibody-containing sample, the cell membranes of the $CD4^+$ cell and the HIV-1 envelope glycoprotein$^+$ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to quantitatively determine the ability of the antibody-containing sample to inhibit the

fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell; and (d) determining whether the antibody-containing sample inhibits the fusion of a first control cell with a second control cell under conditions which would permit non-HIV-1 envelope glycoprotein-mediated fusion of the first and second control cells in the absence of the agent, so as to quantitatively determine the ability of the antibody-containing sample to specifically inhibit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell.

The antibody-containing sample may be any antibody-containing sample. In one embodiment, the antibody-containing sample is a serum sample. In another embodiment, the antibody-containing sample is an IgG preparation. Methods of obtaining an antibody-containing sample are well known to those skilled in the art.

In one embodiment, the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule.

In the preferred embodiment, the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

In another embodiment, the first dye is a fluorescein moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

In one embodiment, the $CD4^+$ cell is a $CD4^+$ HeLa cell. In another embodiment of the subject invention, the HIV-1 envelope glycoprotein$^+$ cell is an HIV-1_{LAI} gp120/gp41$^+$ HeLa cell.

The subject invention further provides a method for quantitatively determining the ability of an antibody-containing sample to inhibit the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises: (a) contacting a predetermined amount of the antibody-containing sample with a suitable amount of the $CD4^+$ cell and a suitable amount of the HIV-1 envelope glycoprotein$^+$ cell under conditions which would permit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell in the absence of the antibody-containing sample, the cell membranes of the $CD4^+$ cell and the HIV-1 envelope glycoprotein$^+$ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to quantitatively determine the ability of the antibody-containing sample to inhibit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell.

In one embodiment, the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule.

In the preferred embodiment, the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

In another embodiment, the first dye is a fluorescein moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

In one embodiment, the $CD4^+$ cell is a $CD4^+$ HeLa cell. In another embodiment of the subject invention, the HIV-1 envelope glycoprotein$^+$ cell is an HIV-1_{LAI} gp120/gp41$^+$ HeLa cell.

The subject invention further provides a method for determining the stage of clinical prognosis of an HIV-1 infection in an HIV-1-infected subject which comprises: (a) obtaining an antibody-containing sample from the HIV-1-infected subject; (b) quantitatively determining the ability of the antibody-containing sample so obtained to inhibit the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell by the method of the subject invention; and (c) comparing the ability of the antibody-containing sample to inhibit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell so determined with that of an antibody-containing sample obtained from an HIV-1 infected subject having an HIV-1 infection at a known stage or having a known clinical prognosis, so as to determine the stage or clinical prognosis of the HIV-1 infection in the HIV-1-infected subject.

As used herein, an "HIV-infected subject" means a subject having at least one of his own cells invaded by HIV-1. In the preferred embodiment, the subject is a human.

The subject invention further provides a method for determining the efficacy of an anti-HIV-1 vaccination in a vaccinated, non-HIV-1-infected subject which comprises: (a) obtaining an antibody-containing sample from the vaccinated, non-HIV-1-infected subject; (b) quantitatively determining the ability of the antibody-containing sample so obtained to inhibit the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell by the method of the subject invention; and (c) comparing the

ability of the antibody-containing sample to inhibit the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell so determined with that of an antibody-containing sample obtained from a vaccinated, non-HIV-1-infected subject for whom the anti-HIV-1 vaccination has a known efficacy, so as to determine the efficacy of the anti-HIV-1 vaccination in the vaccinated, non-HIV-1-infected subject.

As used herein, "anti-HIV-1 vaccination" means the administration to a subject of a vaccine intended to elicit the production of antibodies by the vaccinated subject which are capable of specifically binding to epitopes present on an HIV-1 surface envelope glycoprotein. Vaccines in general are well known to those skilled in the art, and comprise an antigen, e.g., a protein, and an adjuvant.

As used herein, the "efficacy of an anti-HIV-1 vaccination" means the degree to which the vaccination or successive vaccinations (i.e., immunization) causes the titre of HIV-1-neutralizing antibodies in the vaccinated subject to increase. In other words, the higher the efficacy of an anti-HIV-1 vaccination, the higher the titre of HIV-1-neutralizing antibodies in the vaccinated subject.

As used herein, a "non-HIV-1-infected subject" means a subject not having any of his own cells invaded by HIV-1. In the preferred embodiment, the subject is a human.

The subject invention further provides a kit for determining whether an agent is capable of specifically inhibiting the fusion of a CD4⁺ cell with an HIV-1 envelope glycoprotein⁺ cell which comprises, in separate

compartments: (a) a suitable amount of a $CD4^+$ cell whose cell membrane is labeled with a first dye; (b) a suitable amount of an HIV-1 envelope glycoprotein$^+$ cell whose cell membrane is labeled with a second dye, the HIV-1 envelope glycoprotein$^+$ cell being capable of fusing with the $CD4^+$ cell of (a) under suitable conditions in the absence of the agent, and the first and second dyes permitting resonance energy transfer therebetween only when juxtaposed within the same membrane; (c) a suitable amount of a first control cell whose cell membrane is labeled with the first dye; and (d) a suitable amount of a second control cell whose cell membrane is labeled with the second dye, the second control cell being capable of non-HIV-1 envelope glycoprotein-mediated fusion with the first control cell of (c) under suitable conditions in the absence of the agent.

The kit of the subject invention may further comprise additional buffers. Furthermore, the cells may either be dried or suspended in liquid or gel.

The suitable amounts of cells are amounts which would permit one skilled in the art to determine, without undue experimentation, whether an agent is capable of specifically inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell. Such amounts may be readily determined according to methods well known to those skilled in the art.

In one embodiment, the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule.

In the preferred embodiment, the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and

the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

In another embodiment, the first dye is a fluorescein moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

In one embodiment, the $CD4^+$ cell is a $CD4^+$ HeLa cell. In another embodiment of the subject invention, the HIV-1 envelope glycoprotein$^+$ cell is an HIV-1_{LAI} gp120/gp41$^+$ HeLa cell.

The subject invention further provides a kit for determining whether an agent is capable of inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises, in separate compartments: (a) a suitable amount of a $CD4^+$ cell whose cell membrane is labeled with a first dye; and (b) a suitable amount of an HIV-1 envelope glycoprotein$^+$ cell whose cell membrane is labeled with a second dye, the HIV-1 envelope glycoprotein$^+$ cell being capable of fusing with the $CD4^+$ cell of (a) under suitable conditions in the absence of the agent, and the first and second dyes permitting resonance energy transfer therebetween only when juxtaposed within the same membrane.

The kit of the subject invention may further comprise additional buffers. Furthermore, the cells may either be dried or suspended in a liquid or gel carrier.

The suitable amounts of cells are amounts which would permit one skilled in the art to determine, without undue experimentation, whether an agent is capable of inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell. Such amounts may be readily

determined according to methods well known to those skilled in the art.

In one embodiment, the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule.

In the preferred embodiment, the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

In another embodiment, the first dye is a fluorescein moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

In one embodiment, the $CD4^+$ cell is a $CD4^+$ HeLa cell. In another embodiment of the subject invention, the HIV-1 envelope glycoprotein$^+$ cell is an HIV-1_{LAI} gp120/gp41$^+$ HeLa cell.

The subject invention further provides a method for determining whether an HIV-1 isolate is syncytium-inducing which comprises: (a) obtaining a sample of an HIV-1 isolate envelope glycoprotein$^+$ cell whose cell membrane is labeled with a first dye; (b) contacting a suitable amount of the sample with a suitable amount of a $CD4^+$ cell under conditions which would permit the fusion of the $CD4^+$ cell with a syncytium-inducing HIV-1 strain envelope glycoprotein$^+$ cell, the cell membrane of the $CD4^+$ cell being labeled with a second dye which permits resonance energy transfer between the first dye only when the first and second dyes are juxtaposed within the same membrane; (c) determining the percent resonance energy transfer value of the resulting sample after a suitable

period of time; and (d) comparing the percent resonance energy transfer value so determined with a known standard, so as to determine whether the HIV-1 isolate is syncytium-inducing.

As used herein, "syncytium-inducing" means capable of causing the formation of syncytia (multi-nucleated cells resulting from HIV-1 envelope glycoprotein-mediated cell fusion) when contacted with a plurality of $CD4^+$ cells under suitable conditions.

Obtaining a sample of an HIV-1 isolate envelope glycoprotein$^+$ cells may be performed according to methods well known to those skilled in the art.

HIV-1 isolate envelope glycoprotein$^+$ cells may be obtained from blood or any other bodily fluid known to contain HIV-1 isolate envelope glycoprotein$^+$ cells in HIV-infected subjects. Alternatively, HIV-1 isolate envelope glycoprotein$^+$ cells may be obtained by culturing cells _in vitro_ with blood or other bodily fluids containing the HIV-1 isolate or HIV-1 isolate-infected cells, and recovering the HIV-1 isolate envelope glycoprotein$^+$ cells produced thereby.

The suitable amounts of sample and $CD4^+$ cell may be determined according to methods well known to those skilled in the art.

In one embodiment, the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule.

In the preferred embodiment, the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and

the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

In another embodiment, the first dye is a fluorescein moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

In one embodiment, the $CD4^{+}$ cell is a $CD4^{+}$ HeLa cell.

The subject invention further provides a method for determining the stage of an HIV-1 infection in an HIV-1-infected subject which comprises determining by the method of the subject invention whether the HIV-1 isolate with which the HIV-1-infected subject is infected is syncytium-inducing, so as to thereby determine the stage of the HIV-1 infection in the HIV-1-infected subject.

Finally, the subject invention provides a method for quantitatively measuring the fusion of a $CD4^{+}$ cell with an HIV-1 envelope glycoprotein^{+} cell which comprises: (a) contacting a sample of the $CD4^{+}$ cell with the HIV-1 envelope glycoprotein^{+} cell under conditions permitting fusion therebetween, the cell membranes of the $CD4^{+}$ cell and the HIV-1 envelope glycoprotein^{+} cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane; (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to quantitatively measure the fusion of the $CD4^{+}$ cell with the HIV-1 envelope glycoprotein^{+} cell.

This invention will be better understood by reference to

the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

A - Background

The RET-based fusion assay of the subject invention measures fusion between cells which express the HIV-1 envelope glycoprotein (gp120/gp41) and cells which express CD4. Such cell-cell fusion may lead to the production of multinucleated cells or syncytia. Molecules which block HIV-1 attachment or fusion to host cells also block syncytia formation. Syncytia assays have been used in many laboratories to detect virus or anti-virus molecules, and typically have a visual readout. In the development of the assay, permanent cell lines which stably express gp120/gp41 or CD4 were used.

The resonance energy transfer technique has been used in a variety of studies of membrane fusion including the fusion of nucleated cells induced by viruses or polyethylene glycol. However, it has not previously been used to study HIV-1 envelope glycoprotein-mediated membrane fusion. The technique involves labeling one fusion partner (e.g. a gp120/gp41-expressing cell line) with a fluorescent dye such as octadecyl fluorescein (F18) and the other fusion partner (e.g. a CD4-expressing cell line) with a dye such as octadecyl rhodamine (R18). The dyes are chosen such that the emission spectrum of one (F18) overlaps the excitation spectrum of the second (R18). When the cells fuse, the F18 and R18 associate together closely enough that stimulation of F18 results in resonance energy transfer to R18 and emission at the R18 emission wavelengths. The octadecyl versions of the fluors spontaneously insert into the plasma membranes of cells using the labeling protocol described below.

B - Cells Tested

(1) A Chinese Hamster Ovary (CHO) cell line which expresses HIV-1_{IIIB} gp120/gp41 (160G7) was mixed with a human T lymphocyte cell line which expresses CD4 (C8166). $CD4^{+}$ cells are commercially available. 160G7 cells may be obtained at the MRC AIDS Directed Program (United Kingdom). C8166 cells may be obtained at the MRC AIDS Directed Program (United Kingdom) and the NIH AIDS Research and Reference Reagent Program (Bethesda, Maryland). It was previously demonstrated that 160G7 cells and C8166 cells fuse to form multinucleated syncytia. This assay is a syncytium assay which requires visual counting of syncytia with the aid of a low power microscope. This assay is suitable for analyzing blocking agents such as CD4-based molecules and neutralizing antibodies directed against gp120 and gp41.

(2) Human epithelial carcinoma (HeLa) cells which express HIV-1_{LAI} gp120/gp41 (HeLa-env) and HeLa cells which express CD4 (HeLa-$CD4^{+}$) were also used. HeLa-$CD4^{+}$ cells may be obtained at the MRC AIDS Directed Program (United Kingdom) and the NIH AIDS Research and Reference Reagent Program (Bethesda, Maryland). HeLa-env^{+} cells express much higher levels of gp120/gp41 than do 160G7 cells, as demonstrated by the ability to easily detect gp120 on the surface of HeLa-env^{+} cells but not 160G7 cells by flow cytometry using an anti-gp120 antibody. Visual analysis demonstrates that HeLa-env^{+} cells fuse readily with C8166 and HeLa-$CD4^{+}$ cells to form syncytia.

HeLa-env^{+} cells may be obtained, for example, by transfecting HeLa cells with an env-encoding plasmid, such as pMA243, using the calcium phosphate precipitation method and subsequent selection of transfectants with 2μM

methotrexate. The plasmid pMA243 is designed to express the HIV-1_{LAI} genes env, tat, rev and vpu, in addition to the selectable marker DHFR*, with all genes under the control of the HIV-1 LTR (Dragic, T., et al., J. Virol. 66:4794-4802 (1992)). DHFR* is a mutant dihydrofolate reductase gene that demonstrates a reduced affinity for methotrexate. In pMA243, the DHFR* gene is expressed from the mRNA spliced transcript that normally encodes the HIV-1 nef gene which is deleted in this vector. The HIV-1-encoded tat and rev genes are required for high level expression of the env gene. The plasmid pMA243 also encodes an ampicillin resistance marker and bacterial origin of replication.

C - Cuvette Assay Method

The cell labeling conditions were modified from those used in a previous study where RET was used to monitor polyethylene glycol-induced cell fusion (Wanda, P.E., and Smith, J.D., J. Histochem. Cytochem. 30:1297 (1982)). F18 (fluorescein octadecyl ester; Molecular Probes Eugene, Oregon. Catalog No. F3857) or R18 (octadecyl rhodamine B, chloride salt; Molecular Probes, Catalog No. 0246) were dissolved in ethanol at 5-10mg/ml and diluted approximately 1000-fold into the appropriate growth medium. The exact concentration in the medium was adjusted to bring the OD to 0.34 at 506nm (F18) or 1.04 at 565nm (R18). Monolayers of cells were incubated with the appropriate medium overnight, then washed and counted. 100,000 cells of each type were mixed together in wells of a 24-well tissue culture plate. At intervals after mixing, the cells were removed with EDTA, washed and placed in a fluorometer cuvette. Fluorescence was measured at three sets of excitation and emission wavelengths (see table below) using a Perkin-Elmer LS50

fluorometer.

Excitation wavelength	Emission wavelength	measurement obtained
450nm	530nm	Total F18 fluorescence
557nm	590nm	Total R18 fluorescence
450nm	590nm	RET*

* The calculation of RET requires first subtracting the fluorescence due to direct F18 and R18 fluorescence following excitation at 450 and emission at 590. The fluorescence measurements are determined by measuring the fluorescence of cells labeled with each dye separately.

The RET value, calculated as described above, is divided by the total R18 fluorescence to give a % RET value. The results of initial experiments indicate that RET can be measured using both cell combinations listed above. A greater signal was produced when the envelope glycoprotein-expressing cells were F18-labeled and the CD4-expressing cells were R18 labeled than when the envelope glycoprotein-expressing cells were R18-labeled and the CD4-expressing cells were F18 labeled.

D.- <u>Results of time course RET studies and experiments with control cell lines, using the cuvette assay method</u>

Time course experiments were performed with the HeLa-env^{+} + HeLa-CD4^{+} combination (Figure 1). A control cell line, HeLa-Δenv^{+}, was used. HeLa-Δenv^{+} cells express HIV-1 envelope glycoprotein, with a 400 base pair deletion in

the gp120-encoding region of the env gene. These cells do not fuse with CD4$^+$ human cells.

The results demonstrate that fusion can be measured by the RET assay at 2 hours, but not at 1 hour, consistent with previous studies of HIV-1 envelope-mediated cell fusion using fluorescence microscopy. At 4 hours, massive cell fusion was evident by visual inspection of the culture, and this time point yielded reproducible RET values in several experiments. In other experiments, the combination of 160G7 cells with C8166 cells gave a reproducible maximum RET value at about 4 hours but with lower values than those obtained using HeLa-env$^+$ and HeLa-CD4$^+$ (data not shown). Presumably, this difference results from the much greater level of gp120/gp41 expression on HeLa-env$^+$ cells as compared with 160G7 cells.

A number of control experiments were performed using combinations of cells which, based on previous studies, are known not to fuse. These combinations included HeLa cells combined with HeLa-CD4$^+$ cells, or HeLa-env$^+$ cells combined with CHO-CD4 or the human glioma cell line U87.MG-CD4. CHO-CD4 cells, like other non-primate cells, do not fuse with cells expressing HIV-1 gp120/gp41. U87.MG-CD4 cells are one of the few CD4$^+$ human cell lines which do not fuse with HIV-1 envelope glycoprotein-expressing cells. RET values obtained with these combinations of cells (data not shown) were in general similar to those using the control HeLa-Δenv$^+$ + HeLa-CD4$^+$ (Figure 1).

E - <u>Results of RET experiments with blocking agents using the cuvette assay method</u>

It was next determined whether sCD4 (which interacts with gp120/gp41$^+$ cells) or the murine MAb OKT4a (which interacts with CD4$^+$ cells) could block RET (Figures 2 and 3). Both these molecules are known to inhibit HIV-1 infection and syncytium formation. The percent blocking was calculated as % RET at each concentration of blocking agent divided by % RET in the absence of blocking agent at 4 hours.

As shown in Figures 2 and 3, both sCD4 and OKT4a block fusion as measured by RET. The concentrations of these agents required for 50% inhibition are similar to those determined using other assays. For example. the IC_{50} for sCD4 inhibition of fusion between 160G7 an C8166 was approximately 4μg/ml measured using the RET assay, as compared with 5.5μg/ml measured by a visual syncytium assay (i.e., an assay for measuring the inhibition of syncytium formation, wherein the syncytia are quantitated visually using a low-power microscope) using the same combination of cells. In summary, these results demonstrate that the RET method can be used to measure HIV-1 envelope-mediated cell fusion in a rapid and reproducible fashion. When compared with data from the more conventional visual syncytium assay, the results are in excellent agreement.

F - Control blocking experiment with IKT4 using cuvette assay method

Control experiments were performed to examine inhibition of % RET by OKT4. OKT4 is a mouse monoclonal antibody that binds CD4 but does not inhibit the CD4-gp120 interaction, HIV-1 infection, or HIV-induced cell fusion. Using the cuvette method and the HeLa-env$^+$ + HeLa-CD4$^+$ combination, OKT4 gave 0% inhibition of RET at 0.2 μg/ml

or 2.0 μg/ml, compared with 65% inhibition by OKT4a at 0.2 μg/ml in the same experiment. These results demonstrate that inhibition of HIV-1 envelope-mediated membrane fusion as measured by RET is specific for agents that block HIV-1 infection and HIV-induced cell fusion.

G - <u>Automation of the RET assay using the plate reader assay</u>

A fluorescent plate reader was used to analyze the RET assay. This method has the advantage of reducing the manipulations required, notably the need to remove cells for measurement of fluorescence in a cuvette. The plate reader measures fluorescence of cells directly in a multi-well tissue culture plate. Moreover, the speed of assay readout is dramatically increased (by approximately 100-fold). The Millipore "Cytofluor" was used in this experiment. This is a dedicated plate reader which has been used in a variety of different cell-based fluorescence assays and is suitable for use with a range of plate formats including 24-well and 96-well tissue culture plates. The Cytofluor also has the major advantages of speed and compatibility with IBM software analysis programs.

The results indicate that the assay can be readily performed in 24 or 96 well tissue culture plates using the fluorescence plate reader.

In one embodiment, when performing the assay on a routine basis, two types of measurements are done. In the first, RET is measured at a single time point following mixing of labeled cells and a candidate blocking agent. In the second, the assay is adapted to measure changes in the rate of cell fusion in the presence or absence of

blocking agents. One of the advantages of the RET assay is that it measures fusion in real time and thus is amenable to kinetic analysis.

For example, a method of using the plate reader assay and measuring RET at a single time point is provided below. In this assay a 96-well flat bottom tissue culture plate is used. The method is a modification of the cuvette method described above.

Example of a single time-point plate reader assay method:

1. Prepare dyes:
   R18: 10 mg/ml in 100% EtOH (for HeLa-CD4$^+$ cells)
   F18: 5 mg/ml in 100% EtOH (for HeLa-env$^+$ cells)

2. Add dyes to appropriate concentrations, in cell culture medium containing 10% fetal calf serum, as determined by absorbance measurements:
   F18$^+$ medium: 0.34 at 506 nm
   R18$^+$ medium: 0.52 at 565 nm

3. Add medium + dye to the appropriate cells as indicated above, then incubate overnight to stain cells.

4. Wash cells and count.

5. Plate out 20,000 cells of each line/well, some wells having one or other cell line separately, other wells with both cell lines, and other wells with various concentrations of antibodies or other inhibitory agents added in addition to both cell lines.

6. 4 hours later, remove the media and wash all of the wells three times with PBS (the cells remain adherent in the wells). Add 200 μl PBS to each well. Read fluorescence in the wells using the Millipore Cytofluor plate reader with filter combinations listed below:

F18:	excitation 450 nm	emission 530 nm (X)
R18:	excitation 530 nm	emission 590 nm (Y)
F18 + R18:	excitation 450 nm	emission 590 nm (Z)

The emission values, X, Y and Z (as indicated above) are recorded for each cell combination:

A) HeLa-env$^+$ + HeLa-CD4$^+$
B) HeLa-env$^+$ alone
C) HeLa-CD4$^+$ alone

For example, the F18 reading for HeLa-env$^+$ cells alone is given by B_X.

Then % RET is calculated using this formula:

$$\% \text{ RET} = \frac{A_Z - (A_X \cdot B_Z/B_X) - (A_Y \cdot C_Z/C_Y)}{A_Y} \cdot 100$$

Similar results were obtained in experiments comparing inhibition of % RET using the cuvette method and the plate reader method. For example, Figure 4 illustrates the inhibition of fusion between HeLa-env$^+$ and HeLa-CD4$^+$ cells by the monoclonal anti-CD4 antibody, OKT4a, measured as a reduction in % RET determined by both methods at 4 hours after mixing the cells.

What is claimed is:

1. A method for determining whether an agent is capable of specifically inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope $glycoprotein^+$ cell which comprises:
   (a) contacting a sample containing a suitable amount of the agent with a suitable amount of the $CD4^+$ cell and a suitable amount of the HIV-1 envelope $glycoprotein^+$ cell under conditions which would permit the fusion of the $CD4^+$ cell with the HIV-1 envelope $glycoprotein^+$ cell in the absence of the agent, the cell membranes of the $CD4^+$ cell and the HIV-1 envelope $glycoprotein^+$ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane;
   (b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time;
   (c) comparing the percent resonance energy transfer value so determined with a known standard, so as to determine whether the agent is capable of inhibiting fusion of the $CD4^+$ cell with the HIV-1 envelope $glycoprotein^+$ cell; and
   (d) determining whether the agent inhibits the fusion of a first control cell with a second control cell under conditions which would permit non-HIV-1 envelope glycoprotein-mediated fusion of the first and second control cells in the absence of the agent, so as to determine whether the agent is capable of specifically inhibiting the fusion of the $CD4^+$ cell with

the HIV-1 envelope glycoprotein⁺ cell.

2. The method of claim 1, wherein the agent is an antibody.

3. A method for determining whether an agent is capable of specifically inhibiting the infection of a CD4⁺ cell with HIV-1 which comprises determining whether the agent is capable of specifically inhibiting the fusion of a CD4⁺ cell with an HIV-1 envelope glycoprotein⁺ cell by the method of claim 1, so as to thereby determine whether the agent is capable of specifically inhibiting the infection of a CD4⁺ cell with HIV-1.

4. A method for determining whether an agent is capable of inhibiting the fusion of a CD4⁺ cell with an HIV-1 envelope glycoprotein⁺ cell which comprises:

(a) contacting a sample containing a suitable amount of the agent with a suitable amount of the CD4⁺ cell and a suitable amount of the HIV-1 envelope glycoprotein⁺ cell under conditions which would permit the fusion of the CD4⁺ cell with the HIV-1 envelope glycoprotein⁺ cell in the absence of the agent, the cell membranes of the CD4⁺ cell and the HIV-1 envelope glycoprotein⁺ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane;

(b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and

(c) comparing the percent resonance energy transfer

value so determined with a known standard, so as to determine whether the agent is capable of inhibiting fusion of the $CD4^{+}$ cell with the HIV-1 envelope glycoprotein^{+} cell.

5. A method for quantitatively determining the ability of an antibody-containing sample to specifically inhibit the fusion of a $CD4^{+}$ cell with an HIV-1 envelope glycoprotein^{+} cell which comprises:

(a) contacting a predetermined amount of the antibody-containing sample with a suitable amount of the $CD4^{+}$ cell and a suitable amount of the HIV-1 envelope glycoprotein^{+} cell under conditions which would permit the fusion of the $CD4^{+}$ cell with the HIV-1 envelope glycoprotein^{+} cell in the absence of the antibody-containing sample, the cell membranes of the $CD4^{+}$ cell and the HIV-1 envelope glycoprotein^{+} cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane;

(b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time;

(c) comparing the percent resonance energy transfer value so determined with a known standard, so as to quantitatively determine the ability of the antibody-containing sample to inhibit the fusion of the $CD4^{+}$ cell with the HIV-1 envelope glycoprotein^{+} cell; and

(d) determining whether the antibody-containing sample inhibits the fusion of a first control cell with a second control cell under conditions which would permit non-HIV-1

envelope glycoprotein-mediated fusion of the first and second control cells in the absence of the agent, so as to quantitatively determine the ability of the antibody-containing sample to specifically inhibit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell.

6. A method for quantitatively determining the ability of an antibody-containing sample to inhibit the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises:

(a) contacting a predetermined amount of the antibody-containing sample with a suitable amount of the $CD4^+$ cell and a suitable amount of the HIV-1 envelope glycoprotein$^+$ cell under conditions which would permit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell in the absence of the antibody-containing sample, the cell membranes of the $CD4^+$ cell and the HIV-1 envelope glycoprotein$^+$ cell being labeled with a first dye and a second dye, respectively, which first and second dyes permit resonance energy transfer therebetween only when juxtaposed within the same membrane;

(b) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and

(c) comparing the percent resonance energy transfer value so determined with a known standard, so as to quantitatively determine the ability of the antibody-containing sample to inhibit the fusion of the $CD4^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell.

7. A method for determining the stage or clinical prognosis of an HIV-1 infection in an HIV-1-infected subject which comprises:

(a) obtaining an antibody-containing sample from the HIV-1-infected subject;

(b) quantitatively determining the ability of the antibody-containing sample so obtained to inhibit the fusion of a CD4$^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell by the method of claim 6; and

(c) comparing the ability of the antibody-containing sample to inhibit the fusion of the CD4$^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell so determined with that of an antibody-containing sample obtained from and HIV-1-infected subject having an HIV-1 infection at a known stage or having a known clinical prognosis, so as to determine the stage of clinical prognosis of the HIV-1 infection in the HIV-1-infected subject.

8. A method for determining the efficacy of an anti-HIV-1 vaccination in a vaccinated, non-HIV-1-infected subject which comprises:

(a) obtaining an antibody-containing sample from the vaccinated, non-HIV-1-infected subject;

(b) quantitatively determining the ability of the antibody-containing sample so obtained to inhibit the fusion of a CD4$^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell by the method of claim 6; and

(c) comparing the ability of the antibody-containing sample to inhibit the fusion of the CD4$^+$ cell with the HIV-1 envelope glycoprotein$^+$ cell so determined with that of an antibody-

containing sample obtained from a vaccinated, non-HIV-1-infected subject for whom the anti-HIV-1 vaccination has a known efficacy, so as to determine the efficacy of the anti-HIV-1 vaccination in the vaccinated, non-HIV-1-infected subject.

9. A kit for determining whether an agent is capable of specifically inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell which comprises, in separate compartments:

(a) a suitable amount of a $CD4^+$ cell whose cell membrane is labeled with a first dye;

(b) a suitable amount of an HIV-1 envelope glycoprotein$^+$ cell whose cell membrane is labeled with a second dye, the HIV-1 envelope glycoprotein$^+$ cell being capable of fusing with the $CD4^+$ cell of (a) under suitable conditions in the absence of the agent, and the first and second dyes permitting resonance energy transfer therebetween only when juxtaposed within the same membrane;

(c) a suitable amount of a first control cell whose cell membrane is labeled with the first dye; and

(d) a suitable amount of a second control cell whose cell membrane is labeled with the second dye, the second control cell being capable of non-HIV-1 envelope glycoprotein-mediated fusion with the first control cell of (c) under suitable conditions in the absence of the agent.

10. A kit for determining whether an agent is capable of inhibiting the fusion of a $CD4^+$ cell with an HIV-1

envelope glycoprotein$^+$ cell which comprises, in separate compartments:

(a) a suitable amount of a CD4$^+$ cell whose cell membrane is labeled with a first dye; and

(b) a suitable amount of an HIV-1 envelope glycoprotein$^+$ cell whose cell membrane is labeled with a second dye, the HIV-1 envelope glycoprotein$^+$ cell being capable of fusing with the CD4$^+$ cell of (a) under suitable conditions in the absence of the agent, and the first and second dyes permitting resonance energy transfer therebetween only when juxtaposed within the same membrane.

11. A method for determining whether an HIV-1 isolate is syncytium-inducing which comprises:

(a) obtaining a sample of an HIV-1 isolate envelope glycoprotein$^+$ cell whose cell membrane is labeled with a first dye;

(b) contacting a suitable amount of the sample with a suitable amount of a CD4$^+$ cell under conditions which would permit the fusion of the CD4$^+$ cell with a syncytium-inducing HIV-1 strain envelope glycoprotein$^+$ cell, the cell membrane of the CD4$^+$ cell being labeled with a second dye which permits resonance energy transfer between the first dye only when the first and second dyes are juxtaposed within the same membrane;

(c) determining the percent resonance energy transfer value of the resulting sample after a suitable period of time; and

(d) comparing the percent resonance energy transfer value so determined with a known standard, so as to determine whether the HIV-1 isolate is

syncytium-inducing.

12. A method for determining the stage of an HIV-1 infection in an HIV-1-infected subject which comprises determining by the method of claim 11 whether the HIV-1 isolate with which the HIV-1-infected subject is infected is syncytium-inducing, so as to thereby determine the stage of the HIV-1 infection in the HIV-1-infected subject.

13. The method of claim 1, 4, 5, 6, 9, 10 or 11, wherein the first dye is a rhodamine moiety-containing molecule and the second dye is a fluorescein moiety-containing molecule.

14. The method of claim 13, wherein the rhodamine moiety-containing molecule is octadecyl rhodamine B chloride and the fluorescein moiety-containing molecule is fluorescein octadecyl ester.

15. The method of claim 1, 4, 5, 6, 9, 10 or 11, wherein the first dye is a fluorescein moiety-containing molecule and the second dye is a rhodamine moiety-containing molecule.

16. The method of claim 1, 4, 5, 6, 9, 10 or 11, wherein the $CD4^+$ cell is a $CD4^+$ HeLa cell.

17. The method of claim 1, 4, 5, 6, 9 or 10 wherein the HIV-1 envelope $glycoprotein^+$ cell is an $HIV\text{-}1_{LAI}$ $gp120/gp41^+$ HeLa cell.

18. An agent determined to be capable of specifically inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope $glycoprotein^+$ cell using the method of

claim 1.

19. An agent determined to be capable of inhibiting the fusion of a $CD4^+$ cell with an HIV-1 envelope glycoprotein$^+$ cell using the method of claim 4.

FIGURE 1

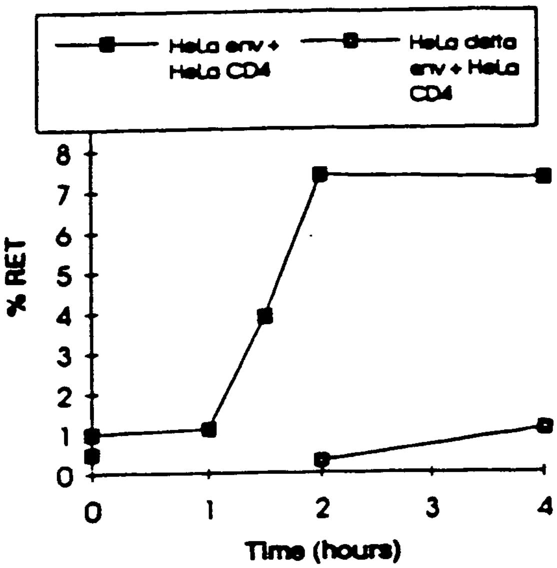
HeLa env + HeLa CD4
HeLa delta env + HeLa CD4
% RET
8
7
6
5
4
3
2
1
0
0
1
2
3
4
Time (hours)

FIGURE 2

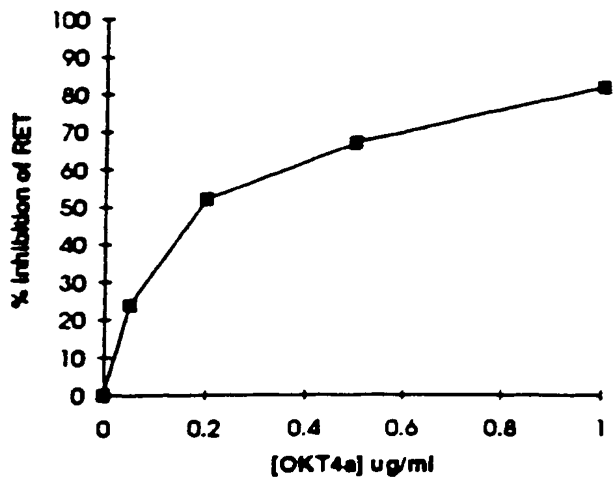
100
90
80
70
60
50
40
30
20
10
0
% Inhibition of RET
0
0.2
0.4
0.6
0.8
1
[OKT4a] ug/ml

FIGURE 3

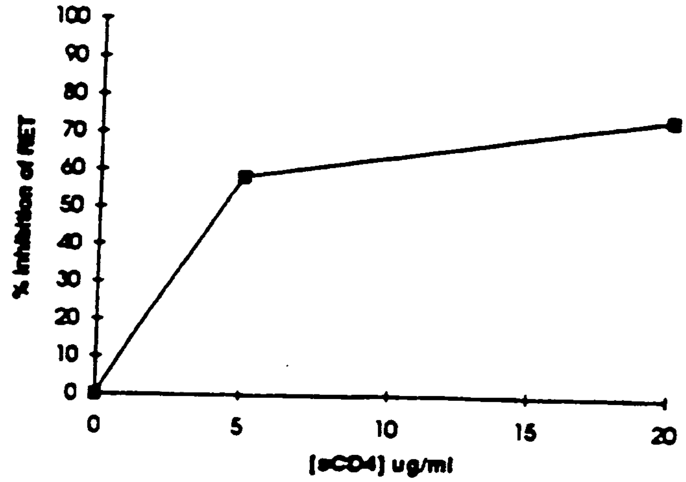
100
90
80
70
60
50
40
30
20
10
0
% Inhibition of RET
0
5
10
15
20
[sCD4] ug/ml

FIGURE 4

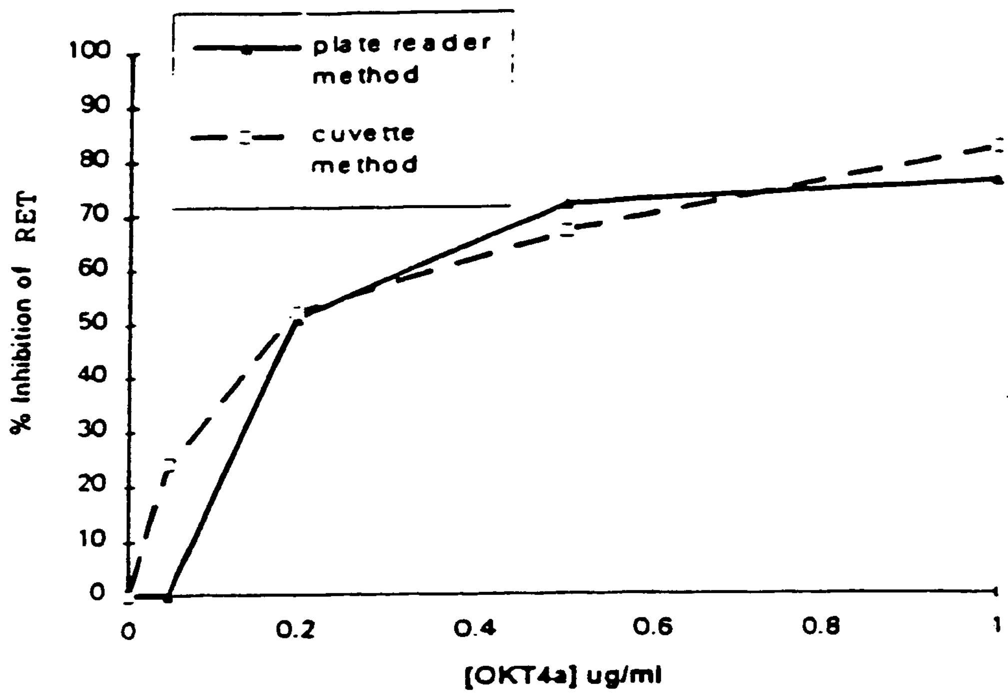

plate reader method
cuvette method
100
90
80
70
60
50
40
30
20
10
0
% Inhibition of RET
0
0.2
0.4
0.6
0.8
1
[OKT4a] ug/ml